THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 56, No. 4

Printed in U.S.A.

ISOLATION AND PROPERTIES OF A GERMINATIVE AND A NON-GERMINATIVE CELL POPULATION FROM POSTEMBRYONIC MOUSE, RABBIT, AND HUMAN EPIDERMIS*

J. T. MOORE, PH.D. AND M. A. KARASEK, PH.D.

ABSTRACT

A method to separate two populations of cells (germinative and non-germinative) from the epidermis of the mouse, rabbit and human is presented. The procedure is both simple and physiological and is based upon the principle that one population of cells (germinative) will selectively attach to a collagen gel while the other (non-germinative) will remain in suspension. The positive identification of each cell population is made by measurement of the extent of incorporation of ^{3}H-thymidine into DNA both *in vivo* and *in vitro*. Morphologic and metabolic studies confirm this identification.

The chemical composition of each cell type has been determined. When compared to the non-germinative cells, the germinative population shows an increase in DNA, RNA, phosphate, and lipids, a decrease in carbohydrates, and an equal quantity of total protein. The germinative cells have three key enzymes in the pathway for DNA synthesis (thymidylate synthetase, thymidine kinase and cytidine diphospho-reductase); the non-germinative cells have no detectable levels of these enzymes.

In 1916 Rous and Jones first reported that suspensions of free cells could be obtained from vertebrate tissue by tryptic digestion (1). Viable cells were obtained from spleen, connective tissue, endothelium, and malignant tumors. Since these initial observations, trypsinization of tissue has been widely used in tissue culture as a method for preparing suspensions of cells from almost all mammalian organs (2) and has been particularly useful in studies of the dissociation, reaggregation and differentiation of chick embryo epidermis (3, 4).

For studies of the biochemistry of epidermal differentiation, uniform populations of cells in the same state of differentiation are required. In attempts to obtain such populations of cells, Giovanella and Heidelberger (5) used trypsinization of mouse skin followed by centrifugation in an albumin gradient. Although some separations based on density were obtained, the low density of serum albumin at maximum concentration did not permit the isolation of discrete bands of basal cells. More recently, Laerum and Boyum (6) used trypsinization to separate the dermis from the epidermis and mechanical scraping of the epidermis to detach basal cells. The viability of the released cells was examined in diffusion chambers implanted intraperitoneally into mice. These studies showed that differentiation and keratinization occurred in some of the surviving cells. The purity of the basal and the differentiated cell suspensions, as determined by the incorporation of thymidine, was 87 percent and 78 percent, respectively.

We have used an observation that following gentle trypsinization of epidermis, epidermal cells with a high nuclear/cytoplasmic ratio selectively attach to a gel prepared from acid-soluble collagen, and have developed this growth characteristic of epidermal cells into a method for the preparation of large quantities of germinative cells and non-germinative cells from the epidermis of the mouse, rabbit, and human. This report describes the technique, the morphology, and some of the chemical and biochemical characteristics of these two cell populations.

MATERIALS AND METHODS

Preparation of epidermal cells. The formation of collagen gels, the dispersion of epidermal cells, and the maintenance of epidermal cells on collagen gels are carried out by methods previously described (7).

Isolation of non-germinative epidermal cells. The methods used in the isolation of the non-germinative cell population are based on the principle that this cell population will not attach

* From the Department of Dermatology, Stanford University School of Medicine, Palo Alto, California 94305.

Received June 25, 1970; accepted for publication November 28, 1970.

Suported in part by Grants AM 10332 and AM 14121, National Institutes of Health, DHEW.

to a collagen gel and will remain in suspension or loosely settled on the surface of a collagen gel.

Twenty-four hours after the plating of epidermal cells on a gel the supernatant solution (containing the non-germinative cells) is removed with a pipette. The surface of the gel is washed 2 times with 5.0 ml of 0.9% NaCl to remove cells settled on the surface of the gel, and the original solution and washes are combined. The non-germinative cells are collected by low-speed centrifugation (5 minutes, 1000 × *g*).

Isolation of germinative epidermal cells. After removal of the non-germinative cell population as described above, the cells that are firmly attached to the collagen gel are incubated with 2.0 ml of a solution of 0.25% trypsin (Difco, 1:250)-1% ethylenediamine tetraacetic acid (EDTA), pH 7.2, for 15 minutes at 37° C. The solution is gently agitated with a Pasteur pipette to detach cells from the gel, and the supernatant solution (containing germinative cells) is removed with a pipette. The surface is washed 2 times with 5.0 ml of 0.9% NaCl, and the washes and original supernatant solution are combined. The cells are collected by low-speed centrifugation (1000 × *g*, 5 minutes) and are washed 2 times with 0.9% NaCl to remove residual trypsin and EDTA.

Cell morphology. The cells in suspension are collected on Nuclepore Membrane Filters (General Electric) and washed gently with 0.9% NaCl (25 ml) and 70% ethanol (25 ml). The filters are removed, placed in Petri dishes, and fixed for a minimum of 1 hour in 70% ethanol. The cells are stained while on the filter with hematoxylin and eosin, and permanent preparations of the stained cells are made by the method of Reynaud and King (8).

Determination of radioactivity. The germinative or non-germinative cells are collected on Whatman glass fiber filter papers (GF/C, 2.4 cm). The filters are rinsed successively with 0.9% NaCl (75 ml), 5% trichloroacetic acid (30 ml) and ether (10 ml). The air-dried filter papers are placed on the bottoms of liquid scintillation vials and the isotope in the cells is solubilized by addition of 0.5 ml of NCS solubilizer (Nuclear-Chicago). After 24 hours at room temperature, 10 ml of liquid scintillation counting solvent (6 ml of 0.4% 2,5 diphenyloxazole-0.05% 1,4-bis [2-(5-phenyloxazoyl)] benzene in toluene) are added, and the vials are equilibrated for 48 hours in the dark prior to the determination of radioactivity in a Nuclear-Chicago liquid scintillation spectrometer.

Preparation of enzyme extracts. Cells are suspended in 0.05 M Tris-HCl buffer, pH 7.6. They are chilled in ice and disrupted by sonic oscillation for 45 seconds (Bronwill "Biosonik") at maximum energy. The disruption is repeated 3 times and verified microscopically. The protein concentration in the extract is determined as described below, and adjusted so that an aliquot contains 0.6–0.8 mg for each enzyme assay.

Chemical determinations.

(a). *DNA.* The diphenylamine method of Dische with dAMP as a standard is used (9). The dAMP concentrations are ranged symmetrically from 12.5 to 100 μM.

(b). *RNA.* The orcinol method of Mejbaum is used with ATP as a standard (10). The ATP concentrations are ranged symmetrically from 10 to 40 μM. dAMP reacts to a negligible extent at concentrations of 12.5 and 25 μM.

(c). *Phosphate.* Total phosphate is determined by the method of Fiske and Subbarow (11). The method of Chen *et al.* (12) is employed to release phosphate from anhydride and/or ester linkage. A standard curve of phosphate is ranged symmetrically from 12.5 to 200 μM.

(d). *Protein.* Protein is determined by the method of Lowry *et al.* (13) with bovine serum albumin as a standard. Protein concentrations are ranged symmetrically from 1 to 10 mg/ml.

(e). *Lipid and phospholipid.* Cell are homogenized and extracted by the method of Tsao and Cornatzer (14). The extract is taken to dryness and redissolved in chloroform-methanol (5 ml; 2:1 v/v). An aliquot (1 ml) is evaporated to dryness under a nitrogen jet and the residue is analyzed for total lipid by the method of Marsh and Weinstein (15). A second aliquot (1 ml) is used to determine phospholipid by the method of Wuthier (16). The range of sensitivity for each of these assays is: 3 to 150 μg for lipid; 0.2 to 3.0 μg for phospholipids.

(f). *Carbohydrate.* Total carbohydrate is determined by the indole reaction of Dische (17). Standard curves of glucose and fructose (5 to 25 μg) are used for calibration.

Enzymatic determinations in germinative and non-germinative cells.

(a). *Thymidylate Synthetase.* Thymidylate synthetase is determined by a modification of the assay system devised by Kammen (18). The total volume of the assay system is 0.2 ml. The system contains in μmoles: formaldehyde, 1.0; DL-tetrahydrofolate (dissolved in 1 M mercaptoethanol), 0.1; Tris-HCl buffer, pH 7.6, 10.0; $MgCl_2$, 4.0; mercaptoethanol, 20.0; and ^{3}HdUMP, 0.02 (5 × 10^6 cpm/μmole).

The enzyme is extracted from cells as described above. Incubations are carried out for 30 minutes at 37° C and terminated by the addition of 0.05 ml of a slurry of Norit A (100 mg/ml in 10^{-3} M phosphate-10^{-3} M pyrophosphate, pH 7.0). After mixing, the suspension is centrifuged for 10 minutes at 800 × *g*. Aliquots of the clear supernatant solution (25 μl) are mixed with methyl cellosolve (3 ml) and scintillation fluor (6 ml of 0.4% 2,5 diphenyloxazole-0.05% 1,4-bis [2-(5-phenyloxazolyl)] benzene in toluene) and counted in a scintillation spectrometer. Synthesis of thymidylate is calculated from the count rates and specific activities of the radioactive substrate.

(b). *Cytidylate reductase.* Cytidylate reductase is determined by a modification of the assay system devised by Turner *et al.* (19). The total volume of the assay system is 0.25 ml. The system contains in μmoles: ATP, 1.7; NaF, 1.6; dithio-

threitol, 1.25; Tris-HCl, pH 7.6, 12.5; $MgCl_2$, 2.0; and ^{3}H-CMP, 0.03 (18 μCi/μmole). Incubations are carried out for 30 minutes at 37° C and terminated by immersing the reaction vessels in a boiling water bath for 2 minutes. Subsequently, 0.1 ml of 1.75 M $HClO_4$ is added and the suspension is maintained at 100° C for 15 minutes to cleave all nucleotides to the monophosphate level. $HClO_4$ is removed by precipitation with KOH. The suspensions are centrifuged for 10 minutes (800 × *g*) and aliquots of the clear supernatant solution (25 μl) are spotted on PEI-cellulose plates (J. T. Baker Chemical Co.). Separation of dCMP and CMP is effected in a borate system described in detail elsewhere (20). The spots corresponding to dCMP are cut out, placed in scintillation vials, and counted as described for thymidylate synthetase.

(c). *Thymidine kinase.* Thymidine kinase is assayed by the method of Bresnick and Thompson (21). The total volume of the assay system is 0.25 ml. The system contains in μmoles: ATP, 1.7; NaF, 1.6; dithiothreitol, 1.25; Tris-HCl, pH 7.6, 12.5; $MgCl_2$, 2.0; and ^{14}C-thymidine, 0.002 (50 μCi/μmole). Incubation conditions are as described for cytidylate reductase. Thymidylate is separated from thymidine on PEI thin-layer chromatographic plates developed in a solvent system containing n-butanol:methanol:water:NH_4OH (60:20:20:1) for 2 hours at ambient temperature.

Incorporation in vivo of nucleic acid and protein precursors into newborn mouse epidermal cells. Each of 7 pairs of newborn mice (1–3 days) is injected subcutaneously with 0.05 ml of one of the following isotopes: ^{14}C-thymidine (58.8 Ci/mole), ^{3}H-histidine (34 Ci/mole), ^{14}C-deoxycytidine (30.4 Ci/mole), ^{3}H-valine (150 Ci/mole), ^{14}C-orotic acid (22.6 Ci/mole) and ^{32}P-phosphate (300 Ci/mole). Two hours after injection the mice are decapitated and exsanguinated, and the skin is removed. The skins are rinsed 3 times in cold physiologic saline containing 200 units penicillin/ml and 100 μg streptomycin/ml. The skin is cut into strips (0.5 cm × 1.0 cm) and epidermal cells are isolated as described previously (7). The cells from each pair of mice are distributed into 4 collagen-coated vials. The germinative and non-germinative cells from 2 vials are pooled and collected, and the activity is determined as described under "Determination of Radioactivity".

Incorporation in vitro of nucleic acid and protein precursors into rabbit epidermal cells. Non-germinative cells from an adult New Zealand rabbit are isolated as described above. The cells are suspended in a complete medium (MEM) and aliquots are placed in vials containing 1.0 μCi of either ^{3}H-thymidine (6700 Ci/mole), ^{14}C-orotic acid (22.6 Ci/mole), ^{3}H-valine (150 Ci/mole), or ^{32}P-phosphate (300 Ci/mole). The cells are incubated for 48 hours at 37° C, the cells are collected, and the incorporation of isotope is determined.

Germinative cells from the same rabbit are fed with a complete medium (MEM) containing 1.0 μCi/ml of the isotopes used for incorporation into non-germinative cells. The cells are incubated for 48 hours at 37° C, are detached from the gel with trypsin-EDTA (see "Isolation of Germinative Cells") and collected, and the incorporation of isotope is determined as described under "Determination of Radioactivity".

RESULTS

Morphology of Germinative and Non-Germinative Epidermal Cells. In Figures 1A and 1B, preparations of newborn mouse skin epidermal cells collected on Nuclepore filters, fixed in ethanol, and stained with hematoxylin and eosin are shown. The germinative cell population (1A) is a uniform population of cells. The cells are basophilic and have a high nuclear/cytoplasmic ratio. As shown in Figure 1B, the non-germinative cell population is a mixed group of cells. It is composed of some injured germinative cells and a spectrum of cells in varying stages of differentiation. The same distribution of cell types is observed in populations of germinative and non-germinative cells isolated from the human and from the rabbit.

Chemical Composition of Germinative and Non-Germinative Cells. The chemical composition of germinative and non-germinative cells is shown in Table I. With the exception of carbohydrate, germinative cells, on a per cell basis, contain more DNA, RNA, phosphate, and lipids than do non-germinative cells. The protein content is equal in both cell types. Carbohydrate is markedly increased in the non-germinative cells. The marked differences in the relative amounts of each constituent are shown in the last column of Table I where the ratios range from 6.7 for phosphate to 0.208 for carbohydrate.

In Table II, a comparison of the content of phosphate in the germinative and non-germinative cells of the mouse, rabbit, and human is presented. Compared to human cells, the mouse germinative or non-germinative cells contain 5-fold as much phosphate. The ratios of phosphate in the germinative/non-germinative cells are similar in all species.

Enzymes of Nucleotide Metabolism in Germinative and Non-Germinative Cells. In Table III, a comparison of the activities of the enzymes thymidine kinase, thymidylate synthetase, and CDP reductase in germinative and non-germinative cells of the mouse epidermis is presented. As seen in this table, no activity for these enzymes is detected in the non-germinative cell population, while each of these enzymes is present in the germinative cell population.

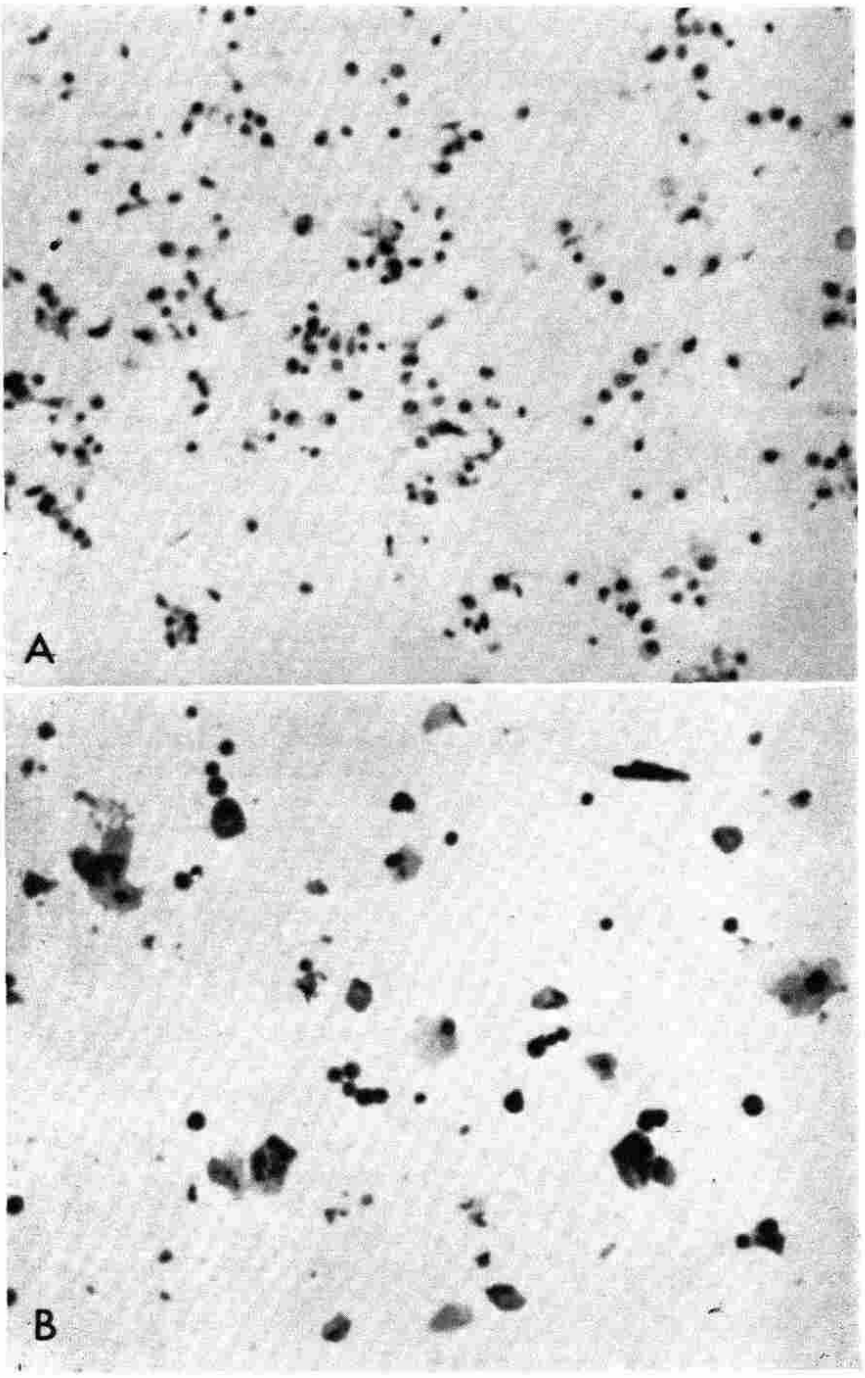

FIG. 1A. Cytology of mouse germinative skin epidermal cells collected on Nuclepore filters, fixed in ethanol, and stained with hematoxylin and eosin. These cells are basophilic and exhibit a high nuclear/cytoplasmic ratio. × 128.

FIG. 1B. Cytology of mouse non-germinative skin epidermal cells collected on Nuclepore filters, fixed in ethanol, and stained with hematoxylin and eosin. These cells are polygonal, eosinphilic, and have a low nuclear/cytoplasmic ratio. × 128.

TABLE I

Chemical composition of germinative and non-germinative newborn mouse epidermal cells

Component	μg/10⁴ Cells		*G/NG
	Germinative	Non-germinative	
$PO_4^{\equiv}$	2.14	0.320	6.7
DNA	0.285	0.071	4.0
RNA	0.341	0.098	3.4
Lipid	3.40	1.67	2.0
Phospholipid	1.88	1.04	1.8
†Protein	0.242	0.240	1.0
Carbohydrate	1.27	6.10	0.2

* Germinative cells/Non-germinative cells.

† Results for protein are expressed as mg/10⁴ cells.

TABLE II

Comparison of the phosphate content of germinative and non-germinative mouse, rabbit, and human epidermal cells

Source of epidermal cells	μmoles $PO_4^{\equiv}$/10⁸ cells		*G/NG
	Germinative	Non-germinative	
Mouse	123.1	18.4	6.7
Rabbit	44.0	11.0	4.0
Human	23.2	4.6	5.0

* Germinative cells/Non-germinative cells.

TABLE III

Enzymes of pyrimidine nucleotide metabolism in germinative and non-germinative newborn mouse epidermal cells

Enzyme	μmoles/30 min./mg protein	
	Germinative cells	Non-germinative cells
Thymidine kinase	3.12	<0.05
Thymidylate synthetase	2.82	<0.05
Cytidine diphospho-reductase	0.00916	<0.0005

Incorporation in Vivo *of Protein and Nucleic Acid Precursors into Mouse Germinative and Non-Germinative Epithelial Cells.* The ability of germinative cells to synthesize DNA and the markedly reduced ability of the non-germinative cells to incorporate precursors of DNA are shown in Table IV. Thymidine and deoxycytidine are incorporated into germinative cells at 13.5 and 19.5 times their rates for incorporation into non-germinative cells. Orotic acid, a precursor of both DNA and RNA, is incorporated 22.5 times faster. The non-germinative cells retain their capacity to synthesize protein as seen by the incorporation of valine and histidine.

Comparison of the Metabolic Activity of Isolated Germinative and Non-Germinative Rabbit Epidermal Cells in Vitro. The ability of isolated epidermal cells to incorporate thymidine, valine, phosphate and orotic acid *in vitro* and the changes in this capacity for non-germinative cells are shown in Table V.

In vitro, rabbit germinative epidermal cells incorporate thymidine 205-fold faster than do non-germinative cells. The rate of incorporation

TABLE IV

Incorporation in vivo *of precursors of protein and nucleic acids into germinative and non-germinative newborn mouse epidermal cells*

Isotope	Radioactivity injected μCi/animal	cpm/10⁶ cells		*G/NG
		Germinative	Non-germinative	
^{14}C-orotic acid	0.50	90	4	22.5
^{3}H-thymidine	1.25	880	45	19.5
^{3}H-deoxycytidine	0.50	400	30	13.4
$^{32}PO_4^{\equiv}$	20.0	3550	564	6.3
^{14}C-valine	5.0	426	463	0.92
^{3}H-histidine	5.0	278	400	0.69

* Germinative cells/Non-germinative cells.

TABLE V

Incorporation in vitro *of precursors of protein and nucleic acids into germinative and non-germinative rabbit epidermal cells*

Isotope	cpm/10⁴ cells		*G/NG
	Germinative	Non-germinative	
^{3}H-thymidine	2058	10	205.8
^{14}C-valine	1235	75	16.5
$^{32}PO_4^{\equiv}$	8853	1406	6.3
^{14}C-orotic acid	108	56	1.93

* Germinative/Non-germinative cells.

of all other precursors, with the exception of phosphate, is decreased.

DISCUSSION

The studies reported in this investigation show that two populations of cells with differences in morphology and biochemistry can be isolated from the skin of the mouse, rabbit, and human. The method is both simple and physiological and can be used to separate large populations of both types of cells. We have named these two cell populations "germinative" and "non-germinative" on the basis of the following observations. When an experimental animal is injected either systemically or subcutaneously with tritiated thymidine, the isotope is incorporated, almost exclusively, into the population of cells labeled "germinative". When this isotope is added to suspensions of the same two cell populations *in vitro* the germinative cells incorporate thymidine while the non-germinative cells do not utilize the isotope. Both cell populations retain the ability to synthesize protein as measured by the incorporation of valine. The non-germinative cells incorporate tritiated histidine to twice the extent of the germinative cells. This finding is in agreement with the known higher rate of incorporation of histidine into non-germinative cells as determined by radioautography (22). Morphologically, the germinative cells are uniform in size and have a high nuclear/cytoplasmic ratio; in contrast, the non-germinative cells are larger, have a lower nuclear/cytoplasmic ratio, and show nuclei in varying stages of hydrolysis. The cytoplasm is more eosinophilic in the non-germinative cells than in the germinative population.

The two cell populations are also unique in their chemistry. Germinative cells contain more RNA, DNA, lipid and phosphate than do non-germinative cells, and the ratios of these constituents in both cell types differ widely. An interesting observation is that the amount of protein is approximately the same in both cell populations but that carbohydrate shows a marked increase in the non-germinative cells (5-fold).

In addition to the chemical changes noted above, the two cell populations differ in their spectrum of enzymatic activities. Of particular interest are three enzymes on the pathway for DNA biosynthesis. No detectable levels of either thymidine kinase, thymidylate synthetase, or CDP reductase are found in the non-germinative cells. Since these cells show little or no ability to incorporate thymidine into nucleic acids, it is interesting to consider that the absence of these enzymes, in particular thymidine kinase, may explain this metabolic difference.

In the past small numbers of basal cells could be isolated from the skin of the mouse (6) but the quantities of cells obtained by these methods were not adequate for quantitative analysis. The procedure described in this report provides a method to obtain larger numbers of two populations of cells in two stages of differentiation. A comparison of the biochemistry of these two cell populations may provide the starting point for a clarification of the metabolic changes that take place during epidermal cell growth and differentiation.

The authors wish to thank Miss Mary Ellen Charlton for excellent technical assistance, and Dr. David I. Wilkinson for the determinations of lipid and phospholipids.

REFERENCES

1. Rous, P. and Jones, F. S.: A method for obtaining suspensions of living cells from the fixed tissues, and for the plating out of individual cells. J. Exp. Med., *23:* 549, 1916.
2. Willmer, E. N.: *Cells and Tissues in Culture: Methods, Biology, and Physiology.* New York, Academic Press, 1965.
3. Moscona, A. A.: Analysis of cell recombinations in experimental synthesis of tissues *in vitro*. J. Cellular Comp. Physiol., *60:* Suppl. 1, 65, 1961.
4. Moscona, A. A.: Studies on stability of phenotypic traits in embryonic integumental tissues and cells, pp. 83–96, *The Epidermis.* Eds., Montagna, W. and Lobitz, W. C. Academic Press, New York, 1964.
5. Giovanella, B. C. and Heidelberger, C.: Mouse epidermal cells and carcinogenesis. I. Isolation of skin constituents. Cancer Res., *25:* 161, 1965.
6. Laerum, O. D. and Boyum, A.: The separation and cultivation of basal and differentiating cells from hairless mouse epidermis. J. Invest. Derm., *54:* 279, 1970.
7. Karasek, M. A. and Charlton, M. E.: Growth and differentiation of post-embryonic skin epithelial cells on collagen gels. J. Invest. Derm., *56:* 205, 1971.
8. Reynaud, A. J. and King, E. B.: A new filter for diagnostic cytology. Acta Cytologica, *11:* 289, 1967.
9. Dische, Z.: Color reactions of nucleic acid components. *The Nucleic Acids: Chemistry and Biology.* Vol. I. Eds., Chargaff, E. and Davidson, J. N., Academic Press, New York, 1955.
10. Schneider, W. C.: Determination of nucleic acids in tissues by pentose analysis. *Methods in Enzymology.* Vol. III. Eds., Colowick, S. P. and Kaplan, N. O., Academic Press, New York, 1957.

11. Fiske, C. H. and Subbarow, Y.: The colorimetric determination of phosphorus. J. Biol. Chem., *66:* 375, 1925.
12. Chen, P. S., Toribara, T. Y. and Warner, H.: Microdetermination of phosphorus. Anal. Chem., *28:* 1756, 1956.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem., *193:* 265, 1951.
14. Tsao, S. S. and Cornatzer, W. E.: Chemical composition of subcellular particles from cultured cells of human tissue. Lipids, *2:* 41, 1967.
15. Marsh, J. B. and Weinstein, D. B.: Simple charring method for determination of lipids. J. Lipid Res., *7:* 574, 1966.
16. Wuthier, R. E.: Two-dimensional chromatography on silica gel-loaded paper for the microanalysis of polar lipids. J. Lipid Res., *7:* 544, 1966.
17. Dische, Z.: P. 79. Ashwell, G.: Colorimetric analysis of sugars. *Methods in Enzymology,* Vol. III. Eds., Colowick, S. P. and Kaplan, N. O., Academic Press, New York, 1957.
18. Kammen, H. O.: A rapid assay for thymidylate synthetase. Anal. Biochem., *17:* 553, 1966.
19. Turner, M. K., Abrams, R. and Lieberman, I.: Meso-α,β-diphenyl succinate and hydroxyurea as inhibitors of deoxycytidylate synthesis in extracts of Ehrlich ascites and L-Cells. J. Biol. Chem., *241:* 5777, 1966.
20. Moore, J. T. and Karasek, M. A.: A rapid thin-layer method for the separation of dCMP from CMP. In preparation.
21. Bresnick, E. and Thompson, U.: Properties of deoxythymidine kinase partially purified from animal tumors. J. Biol. Chem., *240:* 3967, 1965.
22. Fukuyama, K., Nakamura, T. and Bernstein, I.: Differentially localized incorporation of amino acids in relation to epidermal keratinization in the newborn rat. Anat. Rec., *152:* 525, 1965.